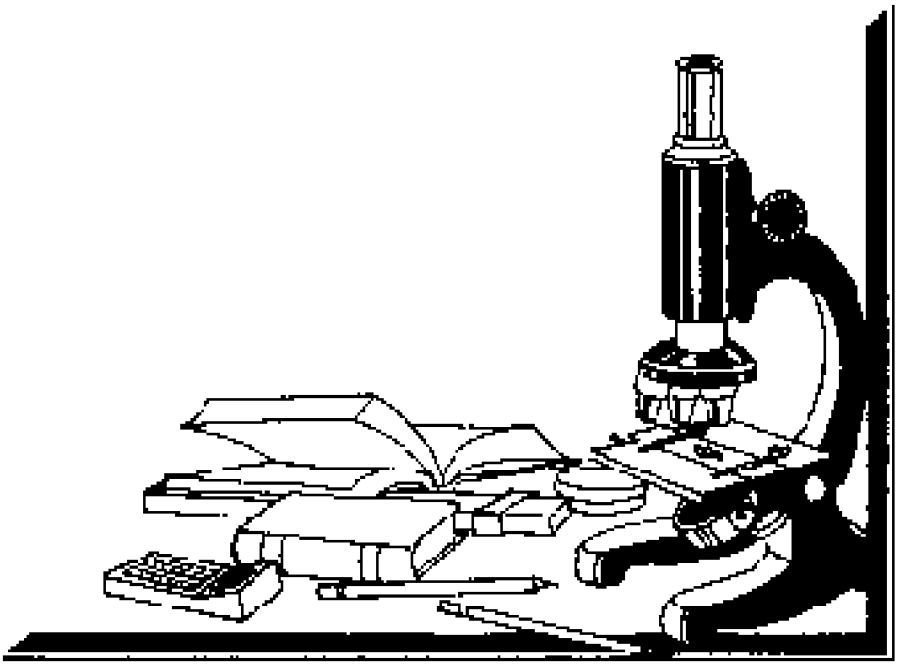
DEPARTMENT OF HEALTH & HUMAN SERVICES

Region VI
Health Care Financing
Administration

1301 Young Street
Room 833
Dallas, Texas 75202

CLIA HANDBOOK FOR CYTOLOGY / HISTOLOGY



Revised March 25, 1998
Gregory Soccio, CT(ASCP)

HCFA
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HCFA REGION VI
BASIC CLIA HANDBOOK FOR
CYTOLOGY / HISTOLOGY

March 25, 1998

Part 1: OBJECTIVE

To assist the laboratory in assessing compliance with the requirements for cytology laboratories regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA).

This handbook **is not** intended to replace the CLIA regulations, or to replace the laboratory's current policies and procedures.

SPECIFIC OBJECTIVES

1. Give a basic overview of the field of cytology.

2. Describe general quality improvement procedures applicable to the practice of cytology.

3. Apply and interpret the prescriptive requirements of the CLIA regulations with respect to the subspecialty of cytology.

Part 2
SURVEY PROCEDURES - LABORATORIES

POLICY FOR CONDUCTING SURVEYS

Survey protocols and Interpretive Guidelines are established pursuant to pertinent sections of the Social Security Act (the Act), Public Health Service Act and 42 CFR Part 493 of the regulations to provide guidance to personnel conducting surveys of laboratories. They clarify and/or explain the intent of the regulations and are required for use by all surveyors assessing laboratory performance based on Federal requirements.

HCFA's mission is to partner with regulated laboratories to help improve patient care and not to promote an antagonistic atmosphere by citing deficiencies that have no direct impact on the laboratory's overall performance. HCFA promotes the use of an educational survey process, rather than one that is punitive in nature. It is the surveyor's objective, using his/her professional judgement, to determine, based on observation of the laboratory's practices, interviews with the laboratory's personnel and review of the laboratory's relevant records, whether it is producing accurate, reliable and timely (quality) test results. The way in which the surveyor meets this objective is by employing an outcome-oriented/quality improvement type of survey process or approach, the intent of which is to focus the surveyor on the overall performance of the laboratory and the way it monitors itself, rather than on a methodical evaluation of each regulatory requirement.

The regulations at 42 CFR Part 493, Subpart Q, permit announced or unannounced surveys. Follow-up or revisit surveys may or may not be announced. Complaint surveys are conducted only on an unannounced basis.

There are several ways to enhance survey effectiveness and efficiency. The surveyor may, except in the case of complaints, consider mailing the appropriate forms to laboratories before the scheduled survey date and request the laboratory to complete:

- o HCFA-1513 Ownership and Control Interest Statement
  (i.e., change of ownership), as needed;
- o HCFA-209 Laboratory Personnel Report
  with directions for completing or updating information;
  adding new personnel or changes in positions or status;
- o HCFA-116 Clinical Laboratory Application
  or state agency form for updating or verifying tests and specialties performed.

The following information should be accessible and retrievable at the time of survey:

- C A list of key facility personnel.
  (Including clerical staff and processing personnel, who will help with obtaining records, etc.)
- C Signature/initial sheet or list of computer ID codes for cytotechnologist and pathologist
- C A copy of cytology classification system, with explanation of diagnostic codes.
- C Review sample of reports for negative, unsatisfactory, abnormal, and non-gynecologic cytology
- C Individual CT workload records (Slides / hours per day)
- C Quality control records
  - Pathologist review (reactive, reparative, atypical, premalignant, malignant)
  - 10% quality control review (including high risk)
  - Retrospective review of previous negatives
  - Cyto / histo correlation
  - Annual statistical evaluation
    - Total volume cases / slides by specimen type
    - # cases reported by diagnosis
    - # cases with discrepant cyto / hist correlation
    - # rescreen reclassified as malignant or premalignant
    - # premalignant or malignant cases where hist is unavailable
- C Equipment Maintenance records
- C Documentation of daily stain checks
- C Quality Assurance documentation of activities
- C Policy and Procedure Manuals
  - Client Service Manual for specimen collection
- C Personnel Files
  - Medical License (Director, Technical Supervisor, Clinical Consultant)
  - Proof of Education (diplomas etc)
  - Proof of Experience (application / resume etc)
  - Assignment of responsibilities (job description etc)
  - Orientation of new employees
  - Documentation of training (processing personnel)
- C List of Reference Laboratories used and their CLIA identification numbers

THE OUTCOME-ORIENTED SURVEY PROCESS

The principal focus of the survey is the effect (outcome) of the laboratories' practices on patient test results and/or patient care. It is our intention to assess those requirements

that will most effectively and efficiently assess the laboratory's ability to provide accurate, reliable and timely test results.

A survey of laboratory services consists of the following elements resulting in an assessment of the principal components listed below:

I. Identifying sources of information
   A. Scheduling surveys
   B. Pre-Survey Preparation

II. Entrance Interview

III. Information gathering
   A. Observation of Facilities and Processes
   B. Interviews
   C. Record review

IV. Assessing Outcome or Potential Outcome

V. Regulatory Compliance Decision

VI. Exit Conference

VII. Formation of the Statement of Deficiencies
   A. Conditions out of Compliance in Patient Test Management, Personnel or Quality Assurance
   B. General Quality Control vs. Specialties/Subspecialties.
   C. Citing Condition-Level Deficiencies in QC

VIII. Survey Report Documentation and Data Input

Part 3
QUALITY ASSURANCE

The purpose of a quality assurance (QA) program is to **monitor and evaluate** the ongoing and overall quality of the total testing process (pre-analytic, analytic, post-analytic).

How is quality assurance different from quality control?

Although an effective quality assurance program will monitor and evaluate the effectiveness of daily quality control, quality assurance **is not** a rehash of quality control.

Much confusion exists in the determining what is quality control (QC) and what is quality assurance (QA). A distinction can be made if you think of QC as something physical that you do to monitor an individual testing process during the analytic phase only. This is in contrast to QA which constantly reviews the policies and procedures for each area of testing to identify potential problems, and identify areas for improvement. Many facilities prefer the terminology Quality Improvement over Quality Assurance. Whatever terminology you use, it is important to recognize the improvement and prevention aspects of quality assurance.

AN EFFECTIVE QUALITY ASSURANCE PROGRAM WILL:

- **Evaluate the effectiveness of policies and procedures;**
- **Identify and correct problems;**
- **Assure the accurate, reliable and prompt reporting of test results; and**
- **Assure the adequacy and competency of the staff.**
- **As necessary, the laboratory must revise policies and procedures based upon the results of those evaluations.**

10 STEPS TO A QUALITY ASSURANCE PROGRAM

1. **EVALUATE SCOPE OF CARE.**
   (What do we do?)

2. **IDENTIFY MAJOR ASPECTS OF CARE.**
   (What is most important?)

3. **DEVELOPE INDICATORS.**
   (What should we monitor?)

4. **ESTABLISH THRESHOLDS.**
   (What is acceptable? AND What is not?)

5. **ASSIGN RESPONSIBILITY.**
   (Who will do the monitoring?)

6. **GATHER DATA & REPORT INFORMATION.**
   (What do we do with all this information?)

7. **TREND ANALYSIS. EVALUATE THE DATA.**
   (Did you find what you expected?)

8. **CORRECTIVE ACTION.**
   (What should we do about it?)

9. **COMMUNICATE INFORMATION.**
   (Does everyone know?)

10. **MONITOR FOR SUSTAINED EFFECTIVENESS.**
    (Did it work?)

ELEMENTS OF A QUALITY ASSURANCE PLAN

PRE-ANALYTICAL

- **PATIENT PREPARATION**
- **SPECIMEN COLLECTION**
- **SPECIMEN ACCEPTABILITY/REJECTION**
- **INTERACTION WITH PHYSICIANS/LABORATORIES REQUESTING THE TEST**

ANALYTICAL

- **TEST ANALYSIS**
- **QUALITY CONTROL**
- **PROFICIENCY TESTING**
- **TURN-AROUND-TIME**
- **REPORTING PANIC VALUES**

POST-ANALYTICAL

- **REPORTING RESULTS**
- **DATA RETRIEVAL**
- **EVALUATION OF Q.C. AND P.T. DATA**
- **REVISE PERFORMANCE CRITERIA**

MANAGERIAL

- **ASSESSMENT OF PERSONNEL COMPETENCY**
- **POLICIES & PROCEDURES**
- **COMPLAINT INVESTIGATIONS**

MANDATORY QUALITY INDICATORS

Each laboratory must establish and follow written policies and procedures for a comprehensive quality assurance program that is designed to monitor and evaluate the ongoing and overall quality of the total testing process (preanalytic, analytic, and postanalytic). As necessary, the laboratory must revise policies and procedures based upon the results its evaluations.

42 CFR 493.1703 Patient test management assessment.

C patient preparation, specimen collection, labeling, preservation and transportation;

C information solicited and obtained on the laboratory's test requisition for its completeness, relevance, and necessity for the testing of patient specimens;

C use and appropriateness of the criteria established for specimen rejection;

C completeness, usefulness, and accuracy of the test report information necessary for the interpretation or utilization of test results;

C timely reporting of test results based on testing priorities (STAT, routine, etc.); and

C accuracy and reliability of test reporting systems, appropriate storage of records and retrieval of test results

42 CFR 493.1705 Quality control assessment.

C problems identified during the evaluation of calibration and control data for each test method;

C problems identified during the evaluation of patient test values for the purpose of verifying the reference range of a test method; and

C errors detected in reported results.

42 CFR 493.1707 Proficiency testing assessment.

C the corrective actions taken for any unacceptable, unsatisfactory, or unsuccessful proficiency testing result(s) must be evaluated for effectiveness.

42 CFR 493.1709 Comparison of test results.

C If a laboratory performs the same test using different methodologies or instruments, or performs the same test at multiple testing sites, the laboratory must have a system that twice a year evaluates and defines the relationship between test results using the different methodologies, instruments, or testing sites.

C If a laboratory performs tests that are not included under Subpart I of this part, Proficiency Testing Programs, the laboratory must have a system for verifying the accuracy of its test results at least twice a year.

42 CFR 493.1711 Relationship of patient information to patients test results.

C For internal quality assurance, the laboratory must have a mechanism to identify and evaluate patient test results that appear inconsistent with relevant criteria such as:

(a) Patient age;
(b) Sex;
(c) Diagnosis or pertinent clinical data, when provided;
(d) Distribution of patient test results when available; and
(e) Relationship with other test parameters, when available within the laboratory.

42 CFR 493.1713 Personnel assessment.

C The laboratory must have an ongoing mechanism to evaluate the effectiveness of its policies and procedures for assuring employee competence and, if applicable, consultant competence.

42 CFR 493.1715 Communications.

C The laboratory must have a system in place to document problems that occur as a result of breakdowns in communication between the laboratory and the authorized individual who orders or receives the results of test procedures or examinations.

42 CFR 493.1717 Complaint investigations.

C The laboratory must have a system in place to assure that all complaints and problems reported to the laboratory are documented. Investigations of complaints must be made, when appropriate, and, as necessary, corrective actions are instituted.

42 CFR 493.1719 Quality assurance review with staff.

C The laboratory must have a mechanism for documenting and assessing problems identified during quality assurance reviews and discussing them with the staff. The laboratory must take corrective actions that are necessary to prevent recurrences.

42 CFR 493.1721 Quality assurance records.

C The laboratory must maintain documentation of all quality assurance activities including problems identified and corrective actions taken. **All quality assurance records must be available to HHS and maintained for a period of 2 years.**

Part 4
OVERVIEW OF THE FIELD OF CYTOLOGY

In the cytology laboratory, specimens from various sites of the body are processed and microscopically examined.

There are four basic "tests" in cytology.

- Diagnosis of malignancy.
- Identification of Premalignant conditions which may progress to malignancy.
- Identification of infections and associated organisms.
- Evaluation of hormonal effects on cell morphology.

1. **Diagnosis of malignancy is the major objective of cytopathology.**

   A malignant tumor is characterized by uncontrolled growth, alterations to varying extent in the structural and functional differentiation of its component cells, and the capacity to spread beyond the limits of the tissue of origin.

2. **Premalignant conditions which may progress to malignancy are identified during the screening process.**

   One of the greatest values of the Pap smear is the ability to detect premalignant changes at the cellular level before these changes are clinically evident. Premalignant conditions may often be successfully eradicated before the process has developed into a life-threatening invasive cancer.

3. **Identification of infections and associated organisms.**

   Pap smears may demonstrate the presence of microorganisms including fungi, protozoa and viruses. The most common fungi detected by Pap smears are Candida albicans, and Torulopsis. Trichomonas vaginalis is the most common protozoan. Herpes simplex and the human papilloma virus (HPV) are the most common viruses detected by the Pap smear. Organisms, including fungi, viruses, and protozoa, may be detected in cytologic specimens from other body sites. Special stains may be used to aid in the detection of these organisms.

4. **Evaluation of hormonal effects on cells.**

The Pap smear may be used to evaluate the effects of estrogen and progesterone on squamous epithelial cells. Squamous epithelial cells mature under the influence of estrogen. Vaginal epithelium provide the most accurate evaluation of hormonal effects. The hormonal evaluation is usually expressed as a ratio of the percentage of cell types counted called a maturation index or MI.

Some clinicians find this information helpful in monitoring replacement hormonal therapy. In post-menopausal women, a high estrogen effect may be associated with tumors of the ovary or uterus. In pregnant women, abnormal hormonal patterns may indicate problems with the pregnancy.

Many factors can influence the accuracy of the MI including endocervical cell contamination, presence of microorganisms or large numbers of inflammatory cells. Patient history, especially menstrual status, and information about medications that the patient is taking at the time of the Pap smear, is very important in providing an accurate hormonal evaluation.

Part 5
WORK FLOW IN A TYPICAL CYTOLOGY LABORATORY

42 CFR 493.1101 CONDITION: PATIENT TEST MANAGEMENT
Each laboratory performing moderate or high complexity testing, or both, must employ and maintain a system that provides for:

- proper patient preparation,
- proper specimen collection,
- identification,
- preservation,
- transportation,
- processing,
- and accurate result reporting.

This system must assure optimum patient specimen integrity and positive identification throughout the pre-analytic (pre-testing), analytic (testing), and post-analytic (post-testing) processes.

For additional resources, refer to NCCLS Guidelines (www.nccls.org/gp.htm)

NCCLS Products

NCCLS940 West Valley Road, Suite 1400
Wayne, PA 19087-1898
phone: 610.688.0100 fax: 610.688.0700
e-mail: exoffice@nccls.org

GP15-A Papanicolaou Technique; Approved Guideline (1994)

- Discusses procedures for cervical specimen collection, as well as the preparation, fixation, staining, and storage of Papanicolaou slides.
- ISBN 1-56238-238-1 Members $25 Nonmembers $85

See videotape section for GP15-A-V information.

A: COLLECTION METHODS

Are instructions available to clients for specimen preservation and transportation?

C Pap Smears
C Body Fluids
C Direct Aspiration

Are the technicians who answer the telephones familiar with the procedures for specimen collection, preservation, and transportation?

NOTE: Approximately 40% of all Pap Smear false negative errors can be contributed to improper collection!

42 CFR 493.1703(a) STANDARD: PATIENT TEST MANAGEMENT ASSESSMENT
The laboratory must monitor, evaluate, and revise, if necessary, based on the results of its evaluations the **criteria established for patient preparation, specimen collection, labeling, preservation, and transportation.**

42 CFR 493.1103(A) STANDARD: SPECIMEN SUBMISSION AND HANDLING
The laboratory must have available and follow written policies and procedures for **specimen collection**.

42 CFR 493.1103(A) STANDARD: SPECIMEN SUBMISSION AND HANDLING The laboratory must have available and follow written policies and procedures for **specimen preservation**.

B: SPECIMEN RECEIVING

Does the technical supervisor ensure that all slides received are matched with the requisition?
What is the laboratory's policy for accepting slides without names on them?
What actions does the technician take when problems are identified?

The Technical Supervisor should periodically observe specimen receiving and processing to assure that the technicians:

- Match all slides with the requisitions;
- Check names and verify any mismatches;
- Check for demographics and patient history;
- Check source of specimen to avoid mistaking a non-gynecologic specimen (i.e. breast smear, or aspirate, etc.) for a pap smear;
- and Record the number of slides received with each requisition.

42 CFR 493.1703(a) STANDARD: PATIENT TEST MANAGEMENT ASSESSMENT The Laboratory must monitor, evaluate, and revise, if necessary, based on the results of its evaluations the criteria established for patient preparation, specimen collection, labeling, preservation and transportation.

42 CFR 493.1103(A) STANDARD: SPECIMEN SUBMISSION AND HANDLING
The laboratory must have available and follow written policies and procedures for **specimen labeling**.

42 CFR 493.1103(A) STANDARD: SPECIMEN SUBMISSION AND HANDLING
The laboratory must have available and follow written policies and procedures for **specimen processing**.

The laboratory's policies and procedures must assure positive identification and optimum integrity of the patient specimens from the time the specimen(s) are collected until testing has been completed and the results reported.

CYTOLOGY SPECIMEN RECEIPT LOG

Date Received	Accession Number	Patient Name	Client	Specimen Source	# Slides	# Blocks

C: CRITERIA FOR SPECIMEN REJECTION

Does the laboratory have specific criteria for specimen rejection?

42 CFR 493.1703(c) STANDARD: PATIENT TEST MANAGEMENT ASSESSMENT The Laboratory must monitor, evaluate, and revise, if necessary, based on the results of its evaluations the use and appropriateness of the criteria established for specimen rejection.

42 CFR 493.1211(B)(1) STANDARD: PROCEDURE MANUAL
The procedure manual must include, when applicable to the test procedure, criteria for specimen rejection.

Specimen Rejection / Problem Log

Date Received	Accession Number	Patient Name	Client	Problem Noted	Action Taken

D: Test Requisition

42 CFR 493.1703(b) STANDARD: PATIENT TEST MANAGEMENT ASSESSMENT
The laboratory must monitor, evaluate, and revise, if necessary, based on the results of its evaluations, the information solicited and obtained on the laboratory's test requisition for its completeness, relevance, and necessity for the testing of patient specimens.

42 CFR 493.1105(E) STANDARD: TEST REQUISITION
The laboratory must assure that the requisition or test authorization includes: (for Pap smears)

- the patient's last menstrual period,
- age or date of birth,
- and indication of whether the patient had a previous abnormal report, treatment or biopsy.

Patient Information (Please Print)

Patient Name _____
Patient I.D.# _____
Age / DOB _____

Physician Name _____
Physician Telephone (___)____-_____

CYTOLOGY, INC.

1301 YOUNG STREET, ROOM 833
DALLAS, TEXAS 75202

Dr. Ding L. Berry, MD, PA

Collection

Date_____

Time _____

#Slides_____

Specimen Source

Cervical _____
Endocervical _____
Endocervical Aspiration _____
Endometrial Aspiration _____
Vaginal _____
OTHER_____

Previous Pap

Date _____

Results _ _____

LMP _____

Clinical Information
(CHECK ALL THAT APPLY)

Pregnant
Cauterization
Post Partum
Cryosurgery
Post Abortion
Laser Surgery
Menopause
Hysterectomy
IUD
Abnormal bleeding
DES
Carcinoma
Oral Contraceptives
Chemotherapy
Exogenous Hormone
Radiation

Additional Clinical Information:
_____
_____
_____
_____
_____
_____
_____

E: Specimen Tracking

How does the laboratory track specimens throughout the entire testing process?
(Pre-analytic, analytic, and post-analytic)

Does the laboratory monitor the requisitions for adequate patient information?

Are ALL pap smears read on site?

42 CFR 493.1703(a) STANDARD: PATIENT TEST MANAGEMENT ASSESSMENT
The Laboratory must monitor, evaluate, and revise, if necessary, based on the results of its evaluations the criteria established for patient preparation, specimen collection, labeling, preservation and transportation.

One method for achieving this requirement is to periodically review a sample of slides, requisitions, reports, and interim test records.

- Start by pulling a random sample of slides from the slide file. (About 2 blocks of 10 or 20 from at least two different months will usually be enough.) Your sample should be large enough to verify quality control but not so large that it is burdensome.

- Have the technician show that they can retrieve the requisition, report, and any interim worksheets used by the laboratory for each sample.

- Use this sample to verify patient identification, demographic information, patient history, and verification of any changes made on technical supervisor review or quality control review.

F: REFERRAL OF SPECIMENS

How does the laboratory track specimens that are referred for consultation with other laboratories?

How does the laboratory track specimens that are sent to other locations for pathologist review?

How does the laboratory track specimens that are sent to branch labs for screening and returned to the main lab for pathologist review?

42 CFR 493.1257(A)(5) STANDARD: CYTOLOGY
The laboratory must assure that all cytology **slide preparations are evaluated on the premises of a laboratory certified to conduct testing in the subspecialty of cytology**.

42 CFR 493.1111 STANDARD: REFERRAL OF SPECIMENS
A laboratory must **refer specimens for testing only to a laboratory possessing a valid certificate** authorizing the performance of testing in the specialty or subspeciality of service for the level of complexity in which the referred test is categorized.

42 CFR 493.1111(A) STANDARD: REFERRAL OF SPECIMENS
The referring laboratory **must not revise results** or information directly related to the interpretation of results provided by the testing laboratory.

42 CFR 493.1111(B) STANDARD: REFERRAL OF SPECIMENS
The referring laboratory must retain or **be able to produce an exact duplicate** of each testing laboratory's report.

42 CFR 493.1111(C) STANDARD: REFERRAL OF SPECIMENS
The authorized person who orders a test or procedure must be notified by the referring laboratory of the **name and address of each laboratory location at which a test was performed.**

Part 6
PRINCIPLE OF THE PAPANICOLAOU STAIN

The Papanicolaou method is a polychrome staining reaction designed to display variations of cellular maturity and metabolic activity. Because intact cells overlap and some appear in three-dimensional configurations, the greatest value of the Papanicolaou staining method is the resultant transparency of the cells.

42 CFR 493.1257(A)(1) STANDARD: CYTOLOGY
The laboratory must assure that all gynecologic smears are stained using a Papanicolaou or modified Papanicolaou staining method.

NOTE: H&E stain is unacceptable for Cervical Pap Smears!

A: BENEFITS OF USING PAPANICOLAOU STAIN

- Clear definition of Nuclear detail
- Differential Counter Staining
- Cytoplasmic Transparency

Daily quality control for the Pap stain must include review of slides to determine:

- if the nuclear detail is sufficient to evaluate the chromatin;
- if the counter stains are adequate to identify cell type; and recognize inflammatory agents such as Candida and Trichomonas, etc; and,
- if cellular transparency is sufficient to focus through multiple layers of cells.

B: FIVE STEPS OF THE PAPANICOLAOU STAIN

- Fixation
- Nuclear Stain
- Cytoplasmic stain
- Clearing
- Mounting and Cover slipping

Example of Staining Schedule

Progressive Papanicolaou Method

Fixation	**95% ethanol.....30 minutes**
Hydration	**50% ethanol.....6 - 10 dips** **Water.....@ 5 minutes**
Nuclear Staining	**Hematoxylin.....2 - 4 minutes** **(Adjust time after first rack, based on QC check)** **Water.....Rinse** **Water.....Rinse** **Water.....Rinse** **Bluing Solution.....1 minute**
Dehydration	**50% ethanol.....6 - 10 dips** **95% ethanol.....6 - 10 dips** **95% ethanol.....6 - 10 dips** **95% ethanol.....6 - 10 dips**
Cytoplasmic Staining	**OG.....3 minutes** **95% ethanol.....6 - 10 dips** **95% ethanol.....Rinse** **(Do not allow slides to sit in ethanol)** **EA-50.....10 minutes** **95% ethanol.....Rinse gently** **95% ethanol.....Rinse gently** **95% ethanol.....1 minute**
Final Dehydration	**100% ethanol.....6 - 10 dips** **100% ethanol.....6 - 10 dips** **100% ethanol.....6 - 10 dips**
Clearing	**Xylene.....6 - 10 dips** **Xylene.....6 - 10 dips** **Xylene.....Until ready to coverslip**

B-1. Fixation

The purpose of a cytologic fixative is to maintain the morphologic characteristics as closely as possible.

An appropriate fixative for cytodiagnostic purposes should;

1. Penetrate cells rapidly,
2. Minimize cell shrinkage,
3. Maintain morphologic integrity,
4. Inactivate autolytic enzymes
5. Replace cell water (dehydrate)
6. Allow permeability of dyes across cell boundaries
7. Permit cell adhesion to a glass surface
8. Be matched to the subsequent staining method used
9. Sterilize the specimen
10. Be reproducible
11. Represent a permanent cellular record.

Cytology specimens should be smeared on slides in a thin, even layer and **immediately** fixed. Immediate fixation of cellular material is an essential first step for accurate cytologic interpretation. It cannot be overemphasized that improperly fixed cell samples may result in inaccurate diagnoses.

Types of fixative.

95% ethanol

Commercial fixatives (spray or dip)

Spray fixatives usually consist of an alcohol base and a waxy substance (carbowax) that provides a thin protective coating for the cells.

Hair spray

Not recommended, but can be used in an emergency.

Substitutes

80% isopropanol (rubbing alcohol)
95% denatured alcohol

SPECIMEN FIXATION CHART

(Specimen Fixation is Imperative for Accurate Results)

Histology Specimens	
Routine Tissue Exams	10% Formalin (If 10% Formalin is not available, contact the Histology laboratory for instructions.)
Bone Marrow Aspirate Smears Tissue specimens for Fat stains Foreign Body	Unfixed (Air Dried)
Immunofluorescence Studies Estrogen, Progesterone, Receptor Studies	Other Fixation (Contact Laboratory)
Cytology Specimens	
Prepared Smears Breast Secretions (Nipple Discharge) Bronchial Brushings Esophageal Brushings Gastric Brushings Lymph Node (Touch Preps) Skin Lesions (Tzanck smears for Herpes) Thyroid Needle Aspirations	Spray Fixative (or 95% Alcohol)
Viscous samples (i.e. bronchial or tracheal specimens, synovial fluids, etc.) Sputum Bronchial washings Esophageal washings	2% Carbowax in 50% ethanol 50% ethanol (if carbowax fixative is not available) (Add Equal Volume to an Aliquot of the specimen)
Pleural fluid Peritoneal fluid Pericardial fluid Cul-de-sac fluid	50% ethanol (Add Equal Volume to an Aliquot of the specimen)
Cerebrospinal fluid	None recommended (deliver to laboratory ASAP)
Special Stains	Other Fixation (Contact Laboratory)

B-2. Nuclear Staining

The Nucleus reflects the reproductive potential of the cell. The size and staining intensity (chromasia) of the nucleus is a critical factor in the evaluation of the cell.

B-3. Cytoplasmic Staining

Enables the cytologist to differentiate between cell types

B-4. Clearing

Renders the cytoplasm transparent
Enables the cytologist to see through layers of cells

B-5: Mounting and Cover slipping

The mounting medium acts as a permanent bond between the slide and the coverslip. The refractive index of the medium must be equal to the refractive index of the slide, coverslip, and the material on the slide.

C: STAIN MAINTENANCE

Proper stain maintenance is critical to the final product in cytology.

Is the stain quality control checked on the day the slides are stained, or on the day of screening?
What criteria is used for adjusting staining times?
How does the laboratory document remedial action?

42 CFR 493.1205(D)(1) STANDARD: TEST METHODS
Reagents, solutions, culture media control materials, calibration materials and other supplies, as appropriate, must be labeled to indicate identity and, when significant, titer, strength or concentration.

42 CFR 493.1205(E)(1) STANDARD: TEST METHODS
Reagents, solutions, culture media, control materials, calibration materials and other supplies must be prepared, stored, and handled in a manner to ensure that reagents, solutions, culture media, controls, calibration materials and other supplies are not used when they have exceeded their expiration date, have deteriorated or are of substandard quality.

42 CFR 493.1218(F)(2) STANDARD: CONTROL PROCEDURES
Each day of use (unless otherwise specified in this subpart), the laboratory must test staining materials for intended reactivity to ensure predictable staining characteristics.

DAILY STAIN MAINTENANCE

Date	QC Accession #	Comments / Corrective Actions	Filter Stains	Change Stains	Change Alcohols	Tech Initials

D: Trouble Shooting for Papanicolaou Stains

- This section is a compilation from many sources and is not intended to be all inclusive.
- Any trouble shooting procedure should follow a logical order.
- Any procedure modification or other remedial action must be documented.

Fixation

- Spray fixatives usually consist of an alcohol base and a waxy substance (Carbowax) that provides a thin protective coating for cells. When there are problems with absorption of hematoxylin, this can often be traced back to inadequate removal of the Carbowax from the smears.

- Some manufacturers of spray fixatives recommend removal of Carbowax with water. However, most sources recommend presoaking in 95% ethanol for 10 to 30 minutes which;
    (1) removes the Carbowax,
    (2) completes the fixation process, and
    (3) prepares the slides for the first hydration step in the Papanicolaou process.

(Hydration prepares the cell sample for the uptake of the water based nuclear dye; dehydration prepares the cell sample for the uptake of counterstain; final dehydration and clearing solutions result in cellular transparency and prepare the cell samples for mounting and Cover slipping)

Nuclear Stain

- If coating fixatives are used, frequent changes of Hematoxylin may be necessary.
- A dull gray appearance or lack of contrasting of the cells indicate fresh stains are needed.
- Water rinses should be changed after each use.
- Heavily chlorinated tap water can bleach out hematoxylin.
- Lot-to-lot variation may be expected with hematoxylin. It may be necessary to experiment with staining times to produce optimum results.

TIP: The most powerful tool for testing Pap stains is also one of the simplest to obtain. Buccal smears are readily available, predictable, and easy to interpret.

1. First collect as many buccal smears as you can get, and immediately fix in 95% ethanol. (The buccal mucosa is similar to vaginal smears for staining purposes)
2. Hydrate and stain through the hematoxylin with varying times beginning with one minute with 1/2 minute intervals.
3. Dehydrate clear and coverslip without running through the counter stain.
4. Examine the slides for **nuclear detail** and determine which formula and times give the best results. Times may need to be adjusted on a daily basis depending on the number of slides run through the stain.

- Periodically check the pH of the tap water following the hematoxylin. Optimum pH for the tap water is @ 7.0. If the tap water is not sufficiently alkaline (pH 7.4 or above), then the staining results may vary significantly.

Counter staining

- Do not allow slides to sit in the alcohol solutions following the OG and EA dyes. The stains may be washed out of the cells if slides are allowed to sit for any length of time.

- Ethanol rinses following OG and EA should be rotated as the ethanol nearest the dye becomes discolored.

Mounting and Cover slipping

- The mounting media must be miscible with the clearing agent to prevent fading of the stains.

- The optical density of the mounting media, slides and coverslips must be equivalent.

OTHER COMMONLY USED STAINS IN PATHOLOGY

Procedure	Purpose
Cytology Stains	
Papanicolaou	**Clear definition of Nuclear Detail, Cytoplasmic Differentiation, and Cytoplasmic Transparency**
Shorr	**Hormonal Evaluation**
Wright-Giemsa	**May be used on a variety of Air-Dried smears. Also used on Bone Marrow smears, and other smears or touch preps for the diagnosis of Lupus Erythematosus, or Leukemia.**
Cytology and Histology Special Stains	
Celestin Blue B	**Nuclear Stain**
Gallocyanin	**Nuclear Stain** **Nissl Granules**
Iron Uptake (Fe+ Reaction)	**Melanin**
Mucicarmine	**Mucin**
Prussian Blue	**Iron, Hemosiderin**
Silver Methenamine	**Fungi**
Sudan Black B	**Lipids**

Histologic Stains	
Hematoxylin and Eosin	**Routine Staining of Histologic Sections**
Oil Red O	**Neutral Fats (i.e. Histiocytes which have phagocytized fats in lipid pneumonia)**
Periodic Acid- Schiff (PAS)	**Carbohydrates (Glycogen, starch, cellulose)** **Mucins in the salivary glands, intestinal tract, bronchial glands, uterus, vagina, prostatic secretions.**
Phosphotungstic Acid	**Cross-striations in Rhabdomyosarcoma**
Silver Stain	**Spirochetes** **Donovan Bodies**

E: CROSS CONTAMINATION CONTROL

42 CFR 493.1257(A)(2) STANDARD: CYTOLOGY
The laboratory must assure that effective measures are taken to prevent cross-contamination between gynecologic and non-gynecologic specimens during the staining process.

42 CFR 493.1257(A)(3) STANDARD: CYTOLOGY
The laboratory must assure that non-gynecologic specimens that have a high potential for cross-contamination are stained separately from other non-gynecologic specimens.

42 CFR 493.1257(A)(3) STANDARD: CYTOLOGY
The laboratory must assure that non-gynecologic specimens that have a high potential for cross-contamination are stained using stains that are filtered or changed following staining.

Part 7
LABORATORY REPORTS

A: LABORATORY REPORTS

How does the lab monitor test reporting?

Who releases the final reports for abnormal cytology/histology?

C How does the laboratory ensure that every smear containing cells exhibiting reactive or reparative changes, atypical squamous or glandular cells, premalignant or malignant, condition is confirmed by the technical supervisor?

What is an electronic signature?

C How does the laboratory ensure that the technical supervisor is the only individual authorized to release his or her electronic signature for reports requiring technical supervisor review?

42 CFR 493.1703(d) STANDARD: PATIENT TEST MANAGEMENT ASSESSMENT
The laboratory must monitor, evaluate, and revise, if necessary, based on the results of its evaluation the completeness, usefulness, and accuracy of the **test report information** necessary for the interpretation or utilization of test results.

42 CFR 493.1107(D) STANDARD: TEST RECORDS
The record system must include the records and **dates of all specimen testing**.

42 CFR 493.1107(D) STANDARD: TEST RECORDS
The record system must include the **identity of the personnel** who performed the test(s).

42 CFR 493.1109(B) STANDARD: TEST REPORT
The test report must indicate the name and address of the laboratory **location at which the test was performed,** the test performed, the test result and, if applicable, the units of measurement.

B: RECORD RETENTION

Is the laboratory able to retrieve test reports for 10 years?

Is the laboratory able to retrieve slides for 5 years?

42 CFR 493.1703(F) STANDARD: PATIENT TEST MANAGEMENT ASSESSMENT
The laboratory must monitor **appropriate storage of records and retrieval of test results.**

42 CFR 493.1109 STANDARD: TEST REPORT
For **pathology**, test reports must be retained for a period of **at least ten years** after the date of reporting.

42 CFR 493.1257(G) STANDARD: CYTOLOGY
The laboratory must retain all **slide preparations for five years** from the date of examination.

42 CFR 493.1257(G) STANDARD: CYTOLOGY
Slides may be loaned to proficiency testing programs, in lieu of maintaining them for this time period, provided the laboratory receives written acknowledgment of the receipt of slides by the proficiency testing program and maintains the acknowledgment to document the loan of such slides.

42 CFR 493.1257(G) STANDARD: CYTOLOGY
Documentation for slides loaned or referred for purposes other than proficiency testing must also be maintained.

42 CFR 493.1257(G) STANDARD: CYTOLOGY
All slides must be retrievable upon request.

42 CFR 493.1259(B) STANDARD: HISTOPATHOLOGY
The laboratory **must retain stained slides at least ten years** from the date of examination and retain specimen blocks at least two years from the date of examination.

42 CFR 493.1259(C) STANDARD: HISTOPATHOLOGY
The laboratory **must retain remnants of tissue** specimens in a manner that assures proper preservation of the tissue specimens **until the portions submitted for microscopic examination have been examined and a diagnosis made**.

RECORD RETENTION

RECORD	RETENTION	REG CITE
Requisition	2 years	42 CFR 493.1105
Instrument Printout	2 years	42 CFR 493.1107
Clinical Lab Reports	2 years	42 CFR 493.1109
Interim or Preliminary Reports	2 years	42 CFR 493.1109
Blood Bank Test records Test reports	5 years *	 42 CFR 493.1107 42 CFR 493.1109
* or 6 months after expiration date, whichever is later, in accordance w/ 21 CFR part 606.160(d)		
Patient Chart and/or Medical Record may be used as: Test requisition Test record Test report		 42 CFR 493.1105 42 CFR 493.1107 42 CFR 493.1109
Pathology reports (including Cytology)	10 years	42 CFR 493.1109
Cytology slides	5 years	42 CFR 493.1257
Histology slides	10 years	42 CFR 493.1259
Histology blocks	2 years	42 CFR 493.1259
Tissue remnants	until portions submitted for microscopic examination have been examined and a diagnosis made	42 CFR 493.1259

C: CLASSIFICATION SYSTEMS

Old Class System	Verbal Classification	Old Cervical Intraepithelial Neoplasia (CIN)	New CIN	Bethesda System
Class I	**Negative**	**Within normal limits - to include reactive and repairative changes**	**Within normal limits - to include reactive and repairative changes**	**Within normal limit - to include reactive and repairative changes**
Class II	**Atypical**	**Atypical consistent with;**	**Atypical consistent with;**	**Atypical squamous (or glandular) cells c undetermined significance (ASCUS / AGUS)**
Class III	**Mild to Moderate Dysplasia**	**CIN Grade 1**	**Low Grade CIN**	**Low grade squamou intraepithelial lesion and HPV related lesions (LSIL)**
Class IV	**Severe dysplasia / carcinoma-in-situ**	**CIN Grade 2-3**	**High Grade CIN**	**High grade squamous intraepithelial lesion (HSIL)**
Class V	**Invasive Cancer**	**Consistent with Invasive Cancer**	**Consistent with Invasive Cancer**	**Malignant lesion**

C1: UNSATISFACTORY SPECIMENS

42 CFR 493.1703(C) STANDARD: PATIENT TEST MANAGEMENT ASSESSMENT
The laboratory must monitor, evaluate, and revise, if necessary, based on the results of it s evaluations the use and appropriateness of the **criteria established for specimen rejection.**

42 CFR 493.1257(A)(4) STANDARD: CYTOLOGY
The laboratory must assure that **diagnostic interpretations are not reported on unsatisfactory smears.**

42 CFR 493.1257(E)(1) STANDARD: CYTOLOGY
The laboratory **report must clearly distinguish specimens or smears, or both, that are unsatisfactory for diagnostic interpretation**.

C2: DESCRIPTIVE DIAGNOSIS

42 CFR 493.1703(D) STANDARD: PATIENT TEST MANAGEMENT ASSESSMENT
The laboratory must monitor, evaluate, and revise, if necessary, based on the results of it s evaluations the completeness, usefulness, and accuracy of the test report **information necessary for the interpretation or utilization of test results.**

42 CFR 493.1257(E)(2) STANDARD: CYTOLOGY
The laboratory report must contain **narrative descriptive nomenclature** for all results.

D: BETHESDA CLASSIFICATION SYSTEM

NOTE: The Bethesda Classification system is included in this handbook for purposes of example only. It is not required under the CLIA regulations.

The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses (TBS) was developed at the National Cancer Institute (NCI) in December 1988 to provide uniform diagnostic terminology that would facilitate communication between the laboratory and the clinician. TBS was designed to be flexible so that it could evolve in response to changing needs in cervical cancer screening as well as to advances in the field of cervical pathology. Subsequently, a second workshop was held in April 1991 to evaluate the impact of TBS in actual practice and to amend and modify it where needed.

The format of TBS report includes:

- An evaluation of specimen adequacy, and
- A descriptive diagnosis.

D1: SPECIMEN ADEQUACY

Four elements constitute the adequacy of the specimen for the detection of abnormalities of the uterine cervix:

1. Patient and specimen identification
2. Pertinent clinical information
3. Technical interpretability
4. Cellular composition and sampling of the transformation zone.

"Satisfactory for evaluation" indicates that the specimen has all of the following:

1. Appropriate labeling and identifying information
2. Relevant Clinical information
3. Adequate numbers of well-preserved squamous epithelial cells
4. Adequate endocervical/transformation zone component (from a patient with a cervix).

"Satisfactory for evaluation but limited by..." indicates that the specimen provides useful information; however, interpretation may be compromised.

1. Lack of pertinent clinical information (age, date of last menstrual period, etc.)
2. Partially obscuring blood, inflammation, thick areas, poor fixation, air-drying, contaminants, etc.(50 to 75% of the epithelial cells).
3. Absence of an endocervical transformation zone component in a patient with a cervix.

"Unsatisfactory ..." designation indicates that the specimen in unreliable for the detection of cervical epithelial abnormalities.

1. Lack of patient identification on the specimen and/or requisition form.
2. A slide that is broken and cannot be repaired.
3. Scant squamous epithelial component (well-preserved squamous epithelial cells covering less than 10% of the slide surface)
4. Obscuring blood, inflammation, thick areas, poor fixation, air-drying, contaminant, etc.(75% of the epithelial cells).

D2: DESCRIPTIVE DIAGNOSIS

Benign Cellular Changes

Infection

- Trichomonas vaginalis
- Fungal organisms (Candida)
- Predominance of coccobacilli
- Actinomyces
- Cellular changes associated with Herpes simplex virus

Reactive changes

- Inflammation (including repair)
- Atrophy with inflammation ("atrophic vaginitis")
- Radiation
- Intrauterine contraceptive device (IUD)

Epithelial Cell Abnormalities

Squamous Cell abnormalities

Atypical squamous cells of undetermined significance(ASCUS):

ASCUS may be qualified as to whether a reactive or neoplastic process is favored.

Low-grade squamous intraepithelial lesion (LGSIL):

LGSIL encompasses all changes of Human papilloma virus (HPV) as well as mild dysplasia/cervical intraepithelial neoplasia level 1(CIN1).

High-grade squamous intraepithelial lesion (HGSIL):

HGSIL encompasses Moderate dysplasia, severe dysplasia, and carcinoma in situ/ CIN 2 and CIN 3.

Squamous cell carcinoma:

Glandular Cell Abnormalities

Endometrial cells, cytologically benign, in a postmenopausal woman
Atypical glandular cells of undetermined significance (AGUS)
Endocervical adenocarcinoma
Endometrial adenocarcinoma
Extrauterine adenocarcinoma
Adenocarcinoma, NOS

Other Malignant Neoplasms

Part 8
QUALITY CONTROL ASSESSMENT

What is Quality Control?

- Quality Control (QC) is a set of procedures which evaluates the testing process and determines the acceptability of a test's results.

- The basis for any quality control program is four-fold.

    1. The control material to be used with each assay;
    2. Frequency of use;
    3. Criteria for the acceptability of patients results; and
    3. Documentation required.

- The quality control program should identify a set of remedial actions that are instituted when control results are unacceptable.

Well then. What is Qualtiy Assurance?

- Quality Assurance (QA) is a system for monitoring and assessment of all phases of laboratory testing. The purpose of the QA policy is to identify potential problems before they occur, or to prevent recurrence of identified problems. (Refer to the chapter on Quality Assurance for more information.)

42 CFR 493.1705 STANDARD: QUALITY CONTROL ASSESSMENT
The laboratory must have an ongoing mechanism to evaluate the corrective actions taken under §493.1219, Remedial action. Ineffective policies and procedures must be revised based on the outcome of the evaluation.

42 CFR 493.1445(E)(5) STANDARD: LABORATORY DIRECTOR RESPONSIBILITIES
The laboratory director must **ensure that the quality control programs are established** and maintained to assure the quality of laboratory services provided and to identify failures in quality as they occur.

42 CFR 493.1451(B)(4) STANDARD: TECHNICAL SUPERVISOR RESPONSIBILITIES
The technical supervisor is **responsible for establishing a quality control program** appropriate for the testing performed and establishing the parameters for acceptable levels of analytic performance and ensuring that these levels are maintained throughout the entire testing process from the initial receipt of the specimen, through sample analysis and reporting of test results.

A: GENERAL QUALITY CONTROL

42 CFR 493.1257 CONDITION: CYTOLOGY
To meet the quality control requirements for cytology, the laboratory must comply with the applicable **requirements in 493.1201 through 493.1221 of this subpart**.

493.1201 THROUGH 493.1221 include the following:

- **A1: Facilities**
- **A2: Reagents / Expiration Dates**
- **A3: Procedure Manual**
- **A4: Equipment Maintenance**

A1: FACILITIES

The laboratory director or technical supervisor should periodically observe the facility for **obvious** safety issues.

42 CFR 493.1445(E)(2) STANDARD: LABORATORY DIRECTOR RESPONSIBILITIES
The laboratory director must ensure that the physical plant and **environmental conditions of the laboratory are appropriate for the testing performed.**

42 CFR 493.1204(A) STANDARD: FACILITIES
The laboratory must be constructed, arranged, and maintained to ensure the space and ventilation necessary for conducting all phases of testing, including the pre-analytic (pre-testing), analytic (testing), and post-analytic (post-testing), as appropriate.

42 CFR 493.1445(E)(2) STANDARD: LABORATORY DIRECTOR RESPONSIBILITIES
The laboratory director must ensure that the physical plant and environmental conditions provide a **safe environment in which employees are protected from physical, chemical, and biological hazards.**

42 CFR 493.1204(B) STANDARD: FACILITIES
Safety precautions must be established, posted, and observed to ensure protection from physical, chemical, biochemical and electrical hazards and biohazardous materials.

A2: REAGENTS / EXPIRATION DATES

The laboratory must utilize test methods, equipment, instrumentation, reagents, materials, and supplies that provide accurate and reliable test results and test reports.

42 CFR 493.1205 (d) STANDARD; TEST METHODS, EQUIPMENT, INSTRUMENTATION, REAGENTS, MATERIALS, AND SUPPLIES.
Reagents, solutions, control materials, calibration materials and other supplies, must be labeled to indicate--

(1) Identity and, when significant, titer, strength or concentration;
(2) Recommended storage requirements;
(3) Preparation and expiration date; and
(4) Other pertinent information required for proper use.

42 CFR 493.1205 (e) STANDARD; TEST METHODS, EQUIPMENT, INSTRUMENTATION, REAGENTS, MATERIALS, AND SUPPLIES.
Reagents, solutions, culture media, control materials, calibration materials and other supplies must be prepared, stored, and handled in a manner to ensure that--

(1) Reagents, solutions, culture media, controls, calibration materials and other supplies are not used when they have exceeded their expiration date, have deteriorated or are of substandard quality.
(2) Components of reagent kits of different lot numbers are not interchanged unless otherwise specified by the manufacturer.

A3: PROCEDURE MANUAL

42 CFR 493.1211 (a)STANDARD; PROCEDURE MANUAL.
A written procedure manual for the performance of all analytical methods used by the laboratory must be readily available and followed by laboratory personnel. Textbooks may be used as supplements to these written descriptions but may not be used in lieu of the laboratory's written procedures for testing or examining specimens.

42 CFR 493.1211(b)STANDARD; PROCEDURE MANUAL.
The procedure manual must include:

- Requirements for specimen collection and processing;
- Criteria for specimen rejection;
- Procedures for microscopic examinations, including the detection of inadequately prepared slides;
- Preparation of slides, solutions, calibrators, controls, reagents, stains and other materials used in testing;
- Control procedures;
- Remedial action to be taken when control results fail to meet the laboratory's criteria for acceptability;
- Pertinent literature references;
- Appropriate criteria for specimen storage and preservation to ensure specimen integrity until testing is completed;
- The laboratory's system for reporting patient results including, when appropriate, the protocol for reporting panic values;
- Criteria for the referral of specimens including procedures for specimen submission and handling as described in Sec. 493.1103.

42 CFR 493.1211 STANDARD; PROCEDURE MANUAL.

- Procedures must be approved, signed, and dated by the director.
- Procedures must be re-approved, signed and dated if the directorship of the laboratory changes.
- Each change in a procedure must be approved, signed, and dated by the current director of the laboratory.
- The laboratory must maintain a copy of each procedure with the dates of initial use and discontinuance.
- These records must be retained for two years after a procedure has been discontinued.

A4: EQUIPMENT MAINTENANCE

42 CFR 493.1215 STANDARD; EQUIPMENT MAINTENANCE AND FUNCTION CHECKS.
The laboratory must perform equipment maintenance and function checks that include electronic, mechanical and operational checks necessary for the proper test performance and test result reporting of equipment, instruments and test systems, to assure accurate and reliable test results and reports.

(a) Maintenance of equipment, instruments, and test systems.

- Perform maintenance as defined by the manufacturer and with at least the frequency specified by the manufacturer; and
- Document all maintenance performed.

B: SIX MAJOR COMPONENTSOF CYTOLOGY QUALITY CONTROL

B1: Technical Supervisor Review
B2: 10% Rescreen
B3: Cytologic/Histologic Correlation
B4: Retrospective Rescreen
B5: Statistics
B6: Workload Records

B1: TECHNICAL SUPERVISOR REVIEW

The individual qualified under 42 CFR 493.1449(b) or (k) who provides technical supervision of cytology must ensure:

42 CFR 493.1257(C)(1) STANDARD: CYTOLOGY
All gynecologic smears interpreted to be showing reactive or reparative changes, atypical squamous or glandular cells of undetermined significance, or to be in the premalignant (dysplasia, cervical intraepithelial neoplasia or all squamous intraepithelial lesions including human papilloma virus associated changes) or malignant category are confirmed by a technical supervisor in cytology.

42 CFR 493.1257(C)(2) STANDARD: CYTOLOGY
All **nongynecologic cytologic preparations are reviewed by the technical supervisor** in cytology.

The **report must be signed to reflect the review** or, if a computer report is generated with signature, it must reflect an electronic signature authorized by the technical supervisor in cytology.

QUALITY CONTROL / PATHOLOGIST REVIEW
DISCREPANCY LOG

Tech: _____ Month/Year: _____

Discrepancy Type: (1) Minor Non-Diagnostic (Inflamatory, ECCs, etc.)
(2) Minor Diagnostic (One level discrepancy)
(3) Major Diagnostic (Two or more level discrepancy)

Accession Number	Tech Diagnosis	Pathologist Diagnosis	Discrepancy Type	Corrective Action	Tech Initials

B2: 10% RESCREEN

The laboratory must establish and follow a program designed to detect errors in the performance of cytologic examinations and the reporting of results that includes:

42 CFR 493.1257(D)(1) STANDARD: CYTOLOGY
Review of slides from at least **10 percent** of the gynecologic cases **interpreted to be negative** for reactive, reparative, atypical, premalignant or malignant conditions as defined in paragraph (c)(1) of this section that are examined by each individual not qualified under 493.1449(b) or (k).

42 CFR 493.1257(D)(1) STANDARD: CYTOLOGY
The review...must be done by a technical supervisor in cytology, a cytology general superviso r qualified under 493.1469, or a cytotechnologist qualified under 493.1483 who has the experience specified in 493.1469(b)(2).

42 CFR 493.1257(D)(1)(I) STANDARD: CYTOLOGY

- The review...must include **negative** cases selected at random... **and** from patients....having a **high probability of developing cervical cancer**,

- records of initial examinations and rescreening results must be available;

- and the review must be completed before reporting patient results on those cases selected.

Quality Control Log

Accession Number	CT Diagnosis	Review Diagnosis	Comments

B3: CYTOLOGIC/HISTOLOGIC CORRELATION

42 CFR 493.1711(E) STANDARD: PATIENT INFORMATION AND TEST RESULTS
For internal quality assurance, the laboratory must have a m echanism to identify and evaluate patient test results that appear inconsistent with relevant criteria such as relationship with other tes t parameters, when available within the laboratory.

The laboratory must establish and follow a program designed to detect errors in the performance of cytologic examinations and the reporting of results.

42 CFR 493.1257(D)(2) STANDARD: CYTOLOGY

- The laboratory must compare clinical information, when available, with cytology reports
- and must compare all malignant and premalignant (as defined in paragraph (c)(1) of this section) gynecology reports with the histopathology report, if available in the laborator y (either on-site or in storage),
- and determine the causes of any discrepancies.

Sampling error
- No ECC on Pap
- No Transition on BX

Dx Error

Unresolved

HISTOLOGY / CYTOLOGY CORRELATION LOG

Discrepancy Codes: (1) Sampling Error (2) Diagnostic Error (3) Unresolved

Date	Histology Accession Number	Histology Diagnosis	Cytology Accession Number	Cytology Diagnosis	Correlation YES/NO	Discrepancy Code	Comments & Corrective Actic

B4: RETROSPECTIVE RESCREEN

42 CFR 493.1705(C) STANDARD: QUALITY CONTROL ASSESSMENT
The mechanism must evaluate and review the effectiveness of corrective actions taken for error s detected in reported results.

42 CFR 493.1257(D)(3) STANDARD: CYTOLOGY
For each patient with a current high grade intraepithelial lesion or above (moderate dysplasia o r CIN-2 or above), the laboratory must ..

C review all normal or negative gynecologic specimens received within the previous five years, if available in the laboratory (either on-site or in storage).

C If significant discrepancies are found that would affect patient care, the laboratory mus t notify the patient's physician and issue an amended report.

QUALITY CONTROL - RETROSPECTIVE RESCREEN REPORT

CURRENT HIGH GRADE LESIONS
WITH PREVIOUS NEGATIVE CYTOLOGY

Patient Name: _____ **Client: _____**

Current Accession Number: _____ **Diagnosis: _____**

Comments:

Original Screener: _____ **Reviewer:_____** **Pathologist: _____**

PREVIOUS CASES

Year + Accession Number	Original Diagnosis	Review Diagnosis	Discrepancy	Comments & Corrective Actions

Discrepancy Codes: **(1) No Discrepancy**
(2) Minor Discrepancy
(3) Major Discrepancy

Affect Patient Care : _____No _____Yes

Action Taken:

- **Case reviewed with Cytotechnologist.**
- **Amended Report Issued _____Date.**
- **Report Called to Referring Physician _____**
- **Quality Assurance Review by All Staff.**
- **Other _____**

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QUALITY CONTROL --- RETROSPECIVE REVIEW LOG

Discrepancy Codes: (1) No Discrepancy (2) Minor Discrepancy (3) Major Discrepancy

Current Accession Number	Current Diagnosis	Previous Accession Number	Previous Diagnosis	Review Diagnosis	Comments & Corrective Action

B5: STATISTICS

42 CFR 493.1711(D) STANDARD: PATIENT INFORMATION AND TEST RESULTS
For internal quality assurance, the laboratory must have a m echanism to identify and evaluate patient test results that appear inconsistent with relevant criteria such as distribution of patient test results when available.

42 CFR 493.1257(D)(4) STANDARD: CYTOLOGY
The laboratory must establish and document an **annual statistical evaluation** of :

- number of cytology cases examined,
- number of specimens processed by specimen type,
- volume of patient cases reported by diagnosis (including the number reported as unsatisfactory for diagnostic interpretation),
- number of gynecologic cases where cytology and available histology are discrepant,
- number of gynecologic cases where any rescreen of a normal or negative specimen results in a reclassification as malignant or premalignant, as defined in paragraph (c)(1) of this section,
- and the number of gynecologic cases for which histology results were unavailable t o compare with malignant or premalignant cytology cases as defi ned in paragraph (c)(1) of this section.

42 CFR 493.1257(F) STANDARD: CYTOLOGY
Corrected reports issued by the laboratory must indicate the basis for correction.

42 CFR 493.1257(D)(5) STANDARD: CYTOLOGY
The laboratory must **evaluate the case reviews of each individual examining slides against the laboratory's overall statistical values**, document any discrepancies, including reasons for th e deviation, and document corrective action, if appropriate.

B6: WORKLOAD RECORDS

42 CFR 493.1257(B)(1) STANDARD: CYTOLOGY
The laboratory is responsible for assuring that each individual engaged in the evaluation of cytology preparations by nonautomated microscopic technique examines no more than 100 slides (one patient per slide, gynecologic or nongynecologic, or both) in a 24 hour period, irrespective of the site or laboratory. **This limit represents an absolute maximum number of slides and is not to be employed as a performance target for each individual.**

42 CFR 493.1257(B)(3) STANDARD: CYTOLOGY
The laboratory is responsible for assuring that **records are maintained** of the total number of slides examined by each individual during each 24 hour period, irrespective of the site or laboratory, and the number of hours each individual spends examining slides in the 24 hour period.

42 CFR 493.1451(C)(6) STANDARD: TECHNICAL SUPERVISOR RESPONSIBILITIES
In cytology, the **technical supervisor** or the individual qualified under 439.1449(k)(2), if responsible for screening cytology slide preparations, **must document the number of cytology slides screened** in 24 hours and the number of hours devoted during each 24-hour period to screening cytology slides.

42 CFR 493.1257(B)(3)(I) STANDARD: CYTOLOGY
The laboratory is responsible for assuring that the maximum number of 100 slides described in paragraph (b)(1) of this section is examined in no less than an 8 hour workday.

42 CFR 493.1257(B)(3)(II) STANDARD: CYTOLOGY
The laboratory is responsible for assuring that for the purposes of establishing workload limits for individuals examining slides by nonautomated microscopic technique on other than an 8 hour workday basis (includes full-time employees with duties other than slide examination and part-time employees), a period of 8 hours must be used to prorate the number of slides that may be examined. Use the formula-- No. of hrs examining slides x 100 divided by 8 to determine maximum slide volume to be examined.

CYTOTECHNOLOGISTS DAILY WORKLOAD

Date: _____ Technologist: _____

Gyn Cases Non-Gyn Cases

From Accession	To Accession	# Cases	# Slides		Accession Number	Specimen Source	# Slides

Hours Screening: _____ Admin / Other : _____

Quality Control Pathologist Review

Accession Number	CT Diagnosis	Review Diagnosis	Comments		Accession Number	Ct Diagnosis	Pathologist Diagnosis	Comments

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CYTOTECHNOLOGIST WEEKLY PERFORMANCE REPORT

	Mon	Tues	Wed	Thu	Fri	Sat	Sun	TOTAL
Date:								
Gyn Cases								
Gyn Slides								
WNL & BCC								
ASCUS/AGUS								
LGSIL								
HGSIL								
UNSAT								
Non-Gyn Cases								
Non_Gyn Slides								
QC Cases								
QC Slides								
Outside Cases								
Outside Slides								
TOTAL CASES								
TOTAL SLIDES								
Hours Screening								
Administrative								
Other								

Part 9
PERSONNEL ASSESSMENT

42 CFR 493.1713 STANDARD: PERSONNEL ASSESSMENT
The laboratory must have an ongoing mechanism to evaluate the effectiveness of its policies an d procedures for **assuring employee competence** and, if applicable, consultant competenc

The laboratory director is responsible for the administration of the laboratory.

The tehcnical supervisor is responsible for the technical oversight of the laboratory.

9A: LABORATORY DIRECTOR RESPONSIBILITIES

42 CFR 493.1445 STANDARD: LABORATORY DIRECTOR RESPONSIBILITIES
The laboratory director is responsible for the **overall operation and administration of the laboratory**, including the employment of personnel who are competent to perform test procedures, record and report test results promptly, accurately and proficiently, and for assuring compliance with the applicable regulations.

The laboratory director, if qualified, may perform the duties of the technical supervisor, clinical consultant, general supervisor, and testing personnel, or delegate these responsibilities to personnel meeting the qualifications under 493.1447, 493.1453, 493.1459, and 493.1487 respectively. If the laboratory director reapportions performance of his or her responsibilities, he or she remains responsible for ensuring that all duties are properly performed.

42 CFR 493.1445(E)(11) The laboratory director must **employ a sufficient number of laboratory personnel** with the appropriate education and either experience or training to provide appropriate consultation, properly supervise and accurately perform tests and report test results in accordance with the personnel responsibilities described in this subpart.

42 CFR 493.1445(E)(12) The laboratory director must ensure that prior to testing patients' specimens, all **personnel have the appropriate education and experience,** receive the appropriate training for the type and complexity of the services offered, and have demonstrated that they can perform all testing operations reliably to provide and report accurate results.

42 CFR 493.1445(E)(13) The laboratory director must ensure that **policies and procedures are established for monitoring individuals** who conduct preanalytical, analytical, and postanalytical phases of testing to assure that they are competent and maintain their competency to process specimens, perform test procedures and report test results promptly and proficiently, and whenever necessary, identify needs for remedial training or continuing education to improve skills.

42 CFR 493.1445(E)(15) The laboratory director must **specify, in writing, the responsibilities and duties** of each consultant and each supervisor, as well as each person engaged in the performance of the preanalytic, analytic, and postanalytic phases of testing, that identifies which examinations and procedures each individual is authorized to perform, whether supervision is required for specimen processing, test performance or result reporting and whether supervisory or director review is required prior to reporting patient test results.

9B: TECHNICAL SUPERVISOR RESPONSIBILITIES

42 CFR 493.1451 STANDARD: TECHNICAL SUPERVISOR RESPONSIBILITIES

The technical supervisor is responsible for the technical and scientific oversight of the laboratory including the procedures for evaluation of the competency of the staff.

Evaluation must include, but are not limited to:

- direct observations of routine patient test performance, including patient preparation, if applicable, specimen handling, processing and testing.
- **monitoring the recording and reporting of test results.**
- **review of intermediate test results or worksheets**, quality control records, proficiency testing results, and preventive maintenance records.
- **direct observation of performance of instrument maintenance** and function checks.
- assessment of test performance through **testing previously analyzed specimens,** internal blind testing samples or external proficiency testing samples.
- assessment of problem solving skills.

9C: CLINICAL CONSULTANT RESPONSIBILITIES

42 CFR 493.1457 STANDARD: CLINICAL CONSULTANT RESPONSIBILITIES
The clinical consultant provides consultation regarding the appropriateness of the testing ordered and interpretation of test results.

9D: CYTOLOGY GENERAL SUPERVISOR RESPONSIBILITIES

42 CFR 493.1471(A) STANDARD: CYTOLOGY SUPERVISOR RESPONSIBILITIES
The cytology general supervisor is responsible for the day-to-day supervision or oversight of the laboratory operation and personnel performing testing and reporting test results.

42 CFR 493.1471(B)(1) STANDARD: CYTOLOGY SUPERVISOR RESPONSIBILITIES
The cytology general supervisor must be accessible to provide on-site, telephone, or electronic consultation to resolve technical problems in accordance with policies and procedures established by the technical supervisor of cytology.

8E: CYTOTECHNOLOGIST RESPONSIBILITIES

42 CFR 493.1485(A) STANDARD: CYTOTECHNOLOGIST RESPONSIBILITIES
The cytotechnologist is reponsible for documenting the slide interpretation results of each gynecologic and nongynecologic cytology case he or she examined or reviewed (as specified in §493.1257(d)).

42 CFR 493.1485(B) STANDARD: CYTOTECHNOLOGIST RESPONSIBILITIES
The cytotechnologist is responsible for documenting, for each 24-hour period, the total number of slides examined or reviewed in the laboratory as well as the total number of slides examined or reviewed in any other laboratory or for any other employer.

42 CFR 493.1485(C) STANDARD: CYTOTECHNOLOGIST RESPONSIBILITIES
The cytotechnologist is responsible for documenting the number of hours spent examining slides in each 24-hour period.

Part 10:
HISTOPATHOLOGY

10A: GROSS EXAMINATION OF TISSUE

Section 493.1461(e) and Section 493.1489(b)(c) Guidelines

The technical supervisor may delegate to individuals qualified under section 493.1489 the responsibility for the physical examination/description, including color, weight, measurement and other characteristics of the tissue; or other mechanical procedures for which a specific written protocol has been developed.

The technical supervisor is ultimately responsible for the diagnosis related to the gross examination and must sign the examination report. The technical supervisor is not required to provide direct onsite supervision but is responsible for the accuracy of all test results reported. All physical examinations/ descriptions of tissue including color, weight, measurement and other characteristics of the tissue; or other mechanical procedures performed in the absence of the technical supervisor by individuals qualified under section 493.1489 must be reviewed within 24 hours by the technical supervisor. All microscopic tissue examinations must be performed by individuals qualified under section 493.1489(b), (l) or (m), of the regulation as appropriate.

42 CFR 493.1259 CONDITION: HISTOPATHOLOGY
To meet the quality control requirements for histopathology, a laboratory must comply with the applicable requirements in 493.1201 through 493.1221 of this subpart and paragraphs (a) through (e) of this section.

493.1201 THROUGH 493.1221 include the following:

Facilities
Reagents
Expiration Dates
Procedure Manual
Specimen Rejection
Equipment Maintenance

42 CFR 493.1259(A) STANDARD: HISTOPATHOLOGY
A control slide of known reactivity must be included with each slide or group of slides for differential or special stains. Reaction(s) of the control slide with each special stain must be documented.

42 CFR 493.1259(B) STANDARD: HISTOPATHOLOGY
The laboratory must retain stained slides at least ten years from the date of examination and retain specimen blocks at least two years from the date of examination.

42 CFR 493.1259(C) STANDARD: HISTOPATHOLOGY
The laboratory must retain remnants of tissue specimens in a manner that assures proper preservation of the tissue specimens until the portions submitted for microscopic examination have been examined and a diagnosis made by an individual qualified under 493.1449(b) or 493.1449(l)(1) of this part.

42 CFR 493.1259(D) STANDARD: HISTOPATHOLOGY
All tissue pathology reports must be signed by an individual qualified as specified in paragraph (c) of the section. If a computer report is generated with an electronic signature, it must be authorized by the individual qualified as specified in paragraph (c) of this section.

493.1259(E) STANDARD: HISTOPATHOLOGY
The laboratory must utilize acceptable terminology of a recognized system of disease nomenclature in reporting results.

APPENDIX A

GLOSSARY OF TERMS
USED IN REPORTING GYNECOLOGICAL CYTOLOGY RESULTS

Air-Drying: Smudgy cells with enlarged nuclei may mimic atypical or dysplastic cells. Caused by poor fixation.

Atrophic Changes: Usually occurs in postmenopasual women with a deficiency of estrogen.

Atypia: Nonspecific term referring to cellular changes. The term may be used to describe conditions of abnormality. Inflammatory atypia usually indicates benign nuclear changes and infiltrating inflammatory cells. The definition varies among laboratories.

Atypical: Refers to any abnormality, synonymous with atypia or abnormal. The definition varies among laboratories.

CIN: Cervical Intraepithelial Neoplasia: Premalignant changes of the cervical squamous epithelium, classified by degree of cell change. CIN is a synonym for cervical squamous dysplasia and squamous intraepithelial lesion.
CIN 1 - Mild dysplasia - Low grade squamous intraepithelial lesion
CIN 2 - Moderate dysplasia - High grade squamous intraepithelial lesion
CIN 3 - Severe dysplasia - High grade squamous intraepithelial lesion

CIS: Carcinoma in situ, preinvasive form of squamous carcinoma.

Dysplasia: An abnormal disordered growth and maturation of cells with characteristic microscopic changes in the epithelium. There are variant degrees of this disorder:

Mild dysplasia -	represents the earliest form of premalignant change of the cervix which may spontaneously disappear or may progress to a more abnormal form.
Moderate dysplasia -	represents an increasing amount of abnormal differentiation and may progress to more signification changes. The disordered growth in moderate dysplasia involves up to two-thirds of the thickness of the epithelium.
Severe Dysplasia -	represents the greatest degree of dysplastic changes and borders on carcinoma in situ. In severe dysplasia and carcinoma in situ, disordered growth of dysplastic cells constitutes most or all of the thickness of the epithelium but does not invade the basement membrane.

Endocervical Cells: Columnar cells that line the endocervical canal and the endocervical glands.

Endocervical Component: Includes endocervical cells, mucin and metaplastic squamous cells.

HPV - Human papillomavirus: A sexually transmitted virus causing genital warts. Various types of HPV are strongly correlated with cervical cancer.

Koilocytosis: Koilocytotic cells have enlarged irregular nuclei with perinuclear halos. These changes are indicative of HPV effect.

Maturation Index: The determination of estrogen effect by the percentage of parabasal, intermediate, and superficial cells present on the smear. Maturation index should only be determined on vaginal smears, preferable from the lateral wall.

Metaplasia: Change from one type of normal cells into another type of normal cells. Transformation of endocervical cells into squamous cells (squamous metaplasia) occurs at the cervical transition zone and is a normal process.

Transition Zone: Squamoculumnar junction or transformation zone. Located near the external cervical os where the squamous epithelium lining of the extocervix meets the endocervical epithelium lining of the endocervical canal.

APPENDIX B
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